UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Tuesday, April 27, 2010

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No.: 83019-G
Product Name: HM 4005 Antimicrobial
DP Barcode: D375052

From: Ian Blackwell, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Through: Karen Hicks, Team Leader
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

To: Velma Noble, PM 31/ Tracy Lantz
Regulatory Management Branch
Antimicrobials Division (7510P)

Applicant: BioSafe, Inc.

FORMULATION FROM LABEL:

Active Ingredient(s):	% by wt.
3-(trihydroxysilyl) propyldimethyloctadecyl ammonium chloride	5.0
Other Ingredient(s):	95.0
Total:	100.0

I BACKGROUND: BioSafe, Inc., has submitted a set of five acute toxicity studies to support the data requirements for their pending product, "HM 4005 Antimicrobial". Stillmeadow, Inc., conducted these studies. The registrant wishes to employ the Cite-All method of data support for the dermal sensitization study.

The Product Science Branch (PSB) /Antimicrobials Division (AD) contractor, Computer Sciences Corporation (CSC) Systems and Solutions, LLC, conducted a primary review of these studies. The Chemistry and Toxicology Team (CTT) conducted a brief secondary review to assure that the studies meet EPA/OPP criteria.

II RECOMMENDATIONS:

1. The five studies are acceptable.

2. CTT considers there to be a data gap for the dermal sensitization requirement. The registrant must adequately address the requirement for the dermal sensitization study. This may be done by:

   A. Having a dermal sensitization study conducted on 83019-G.
   B. Citing a dermal sensitization study on a Substantially Similar product. CTT and PM Team 31 have already conducted a search for a Substantially Similar product with a dermal sensitization study, but, could not locate one. BioSafe would have to locate a specific Substantially Similar product with acceptable data on its own.
   C. Requesting a waiver of the dermal sensitization study. This waiver must be based upon the Agency's directives as published in the 40 CFR §161.340. However, it seems highly unlikely that the registrant could receive such a waiver as it does not meet the following waiver criteria.

      1. The product must be a gas or highly volatile. This product is a liquid.
      2. Test material is corrosive to skin or has a pH below 2 or above 11.5. This product is toxicity category IV for primary skin irritation and has a reported pH between 2 and 11.5.
      3. Dermal exposure does not occur under conditions of use. It is rare that CTT or OPP grants a waiver of the dermal sensitization study based upon conditions of use. As HM 4005 is marketed for use in home, office, automobiles, and many other direct applications, it is inconceivable that a waiver of the dermal sensitization study would be granted for this product.

The acute toxicity profile for File Symbol 83019-G is currently:

Study	MRID Number	Toxicity Category	Study Status
Acute Oral Toxicity	479841-01	IV	Acceptable
Acute Dermal Toxicity	479841-02	IV	Acceptable
Acute Inhalation Toxicity	479841-03	IV	Acceptable
Primary Eye Irritation	479841-04	II	Acceptable
Primary Skin Irritation	479841-05	IV	Acceptable
Dermal Sensitization	None	?	Data Gap

III LABELING:

1. The Signal Word is "WARNING" based upon the results of the primary eye irritation study.

2. As one of the six acute toxicity data requirements is unmet, CTT cannot prescribe Precautionary statements

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 31
MRID No.: 479841-04

Reviewer: CSC and Ian Blackwell
Completion Date: February 2, 2010
Study No.: 13531-09

Testing Laboratory: STILLMEADOW, Inc., Sugar Land, TX
Author: Janice O. Kuhn, PhD, DABT

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was designed and performed at STILLMEADOW, Inc. and was conducted in compliance with U.S. EPA FIFRA 40 CFR 160, with the following exception: Characterization and stability information was not provided to the testing facility, as per Section 160.31(d) and 160.105(a)(b)(e).

Test Material: HM 4005 Antimicrobial
Batch #: GDG-071 / Clear water-like liquid

Dosage: Limit Test: 5,000 mg/kg (administered as received)

Species: 3 Rats; Sprague-Dawley, albino
Sex: Females. Females were nulliparous and non-pregnant.
Age: Young adult (11 weeks old)
Weight: 185-224 grams; at experimental start (i.e., Day 0)
Source: Texas Animal Specialties, Humble, TX
Housing: Temperature Range: 19-24°C
Humidity Range: 23-76%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 5 days

Conclusion:

1. **Acute Oral LD_{50} (mg/kg)**: Female Rats: >5,000 mg/kg

2. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1100):

- The laboratory reported the following protocol deviation: "Relative humidity was outside protocol range but did not affect study outcome."
- The guidelines state that the animals are to be observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours, and daily thereafter. The laboratory did not specify the times that the animals were observed after dosing on the day of

treatment, but indicated that observations were made at least three times on the day of dosing and at least once daily thereafter for 14 days.

Results:

Limit Test

Dosing Sequence	Animal No.	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	51	5,000	S	S
2	52	5,000	S	S
3	53	5,000	S	S

S – Survival

Observations:

No mortality occurred during the study. All animals exhibited weekly weight gain during the study. Clinical signs included respiratory chirp, activity decrease, and piloerection. All animals were asymptomatic by Day 4.

Gross Necropsy Findings:

The gross necropsy conducted at termination of the study revealed no observable abnormalities.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 31
MRID No.: 479841-02

Reviewer: CSC and Ian Blackwell
Completion Date: February 9, 2010
Study No.: 13532-09

Testing Laboratory: STILLMEADOW, Inc., Sugar Land, TX
Author: Janice O. Kuhn, PhD, DABT

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was designed and performed at STILLMEADOW, Inc. and was conducted in compliance with U.S. EPA FIFRA 40 CFR 160, with the following exception: Characterization and stability information was not provided to the testing facility, as per Section 160.31(d) and 160.105(a)(b)(e).

Test Material: HM 4005 Antimicrobial,
Batch #: GDG-071 / Clear water-like liquid

Dosage: 5,050 mg/kg (applied undiluted)

Species: 10 Rats; Sprague-Dawley, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (8 weeks old)
Weight: Males: 265-293 grams; Females: 178-188 grams; on day of dosing
Source: Texas Animal Specialties, Humble, TX
Housing: Temperature Range: 21-24°C
Humidity Range: 32-76%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 5 days

Summary:

1. **Acute Dermal LD_{50} (mg/kg)**: Male and Female Rats: >5,050 mg/kg

2. **The estimated acute dermal LD_{50} is** greater than 5,050 mg/kg in male and female rats.

3. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1200):

- The laboratory reported the following protocol deviation: "Relative humidity was outside protocol range but did not affect study outcome."

Results:

Reported Mortality

Dose Level (mg/kg)	Number Dead / Number Tested		
	Males	Females	Total
5,050	0 / 5	0 / 5	0 / 10

Observations:

No mortality occurred during the study. Animals exhibited weekly weight gain during the study. All animals appeared normal for the duration of the study. There were no signs of dermal irritation at any observation during the study.

Gross Necropsy Findings:

The gross necropsy conducted at termination of the study revealed no observable abnormalities.

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)
(NOSE-ONLY EXPOSURE)

Product Manager: 31
MRID No.: 479841-03

Reviewer: CSC and Ian Blackwell
Completion Date: January 26, 2010
Study No.: 13533-09

Testing Laboratory: STILLMEADOW, Inc., Sugar Land, TX
Author: Andrew Doig, M.S.

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was designed and performed at STILLMEADOW, Inc. and was conducted in compliance with U.S. EPA FIFRA 40 CFR 160, with the following exception: Characterization and stability information was not provided to the testing facility, as per Section 160.31(d) and 160.105(a)(b)(e).

Test Material: HM 4005 Antimicrobial
Batch #: GDG-071 / Clear water-like liquid

Species: 10 Rats; Sprague-Dawley, albino
Sex: 5 Males and 5 Females. Females were nulliparous and non-pregnant.
Age: Young adult (10 weeks old)
Source: Texas Animal Specialties, Humble, TX
Weight: Males: 303-322 grams; Females: 180-221 grams; when tested (i.e., Day 0)
Housing: Temperature Range: 19-23°C
Humidity Range: 23-56%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 5 days

Concentration:

Group	Gravimetric Exposure Concentration (mg/L)	Nominal Concentration (mg/L)
I	2.21	23.03

Summary:

1. **LC_{50} (mg/L) 4-hr exposure**: >2.21 mg/L in male and female rats

2. **The estimated 4-hr acute inhalation LC_{50} of HM 4005 Antimicrobial is greater than 2.21 mg/L in male and female rats.**

3. **Average MMAD**: 2.6 µm
4. **Toxicity Category**: IV **Classification**: Acceptable

Procedure (Deviations from 870.1300):

- The laboratory reported the following protocol deviation: "Temperature and humidity was outside protocol range, but did not affect study outcome."
- The guidelines state that the purity of the test substance should be reported. Only the name and lot number of the test substance were provided.

Results:

Reported Mortality

Exposure Concentration (mg/L)	Number Dead / Number Tested		
	Males	Females	Combined
2.21	0 / 5	0 / 5	0 / 10

Chamber Atmosphere

Exp. Conc. (mg/L)	Sample	MMAD (µm)	GSD (µm)	Cumulative % of Particles > Effective Cutoff Diameter (µm)[1]							
				0.3	0.5	0.9	1.6	2.5	4.2	10.4	17.4
2.21	1	2.4	5.1	97.1	91.3	65.2	49.2	34.7	20.2	11.5	5.08
	2	2.7	5.8	92.4	81.1	66.0	43.4	33.9	20.7	11.3	5.66

[1]Percent of particles greater than corresponding effective cutoff diameter

Chamber Environment During Exposure

Exposure Level (mg/L)	2.21
Chamber Volume (L)	500
Average Total Airflow Volume (Lpm)	184
Air Changes Per Hour	22.08
Mean Oxygen Content (%)	at least 19%
Temperature Range (°C)	19.7-20.3
Relative Humidity Range (%)	38.7-42.9

Clinical Observations:

No mortality occurred during the study. Animals exhibited weekly weight gain during the study. Prominent in-life observations included piloerection and activity decrease. Animals were asymptomatic by Day 6.

Gross Necropsy Findings:

The gross necropsy conducted on each animal at termination of the study revealed no observable abnormalities.

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 31
MRID No.: 479841-04

Reviewer: CSC and Ian Blackwell
Completion Date: February 9, 2010
Study No.: 13534-09

Testing Laboratory: STILLMEADOW, Inc., Sugar Land, TX
Author: Janice O. Kuhn, PhD, DABT

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was designed and performed at STILLMEADOW, Inc. and was conducted in compliance with U.S. EPA FIFRA 40 CFR 160, with the following exception: Characterization and stability information was not provided to the testing facility, as per Section 160.31(d) and 160.105(a)(b)(e).

Test Material: HM 4005 Antimicrobial
Batch #: GDG-071 / Clear water-like liquid

Dosage: 0.1 mL (instilled undiluted)

Species: 3 Rabbits; New Zealand White, albino
Sex: 2 Males and 1 Female. Female was nulliparous and non-pregnant.
Age: Adult (15 weeks old)
Weight: Males: 2.750-3.275 kilograms; Female: 2.500 kilograms; initial body weight
Source: Nichols Rabbitry Inc., Lumberton, TX
Housing: Temperature Range: 18-22°C
Humidity Range: 23-91%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 5 days

Summary:

1. **Toxicity Category**: II (moderately irritating)
2. **Classification**: Acceptable

Procedure (Deviations from 870.2400):

- The laboratory reported the following protocol deviation: "Relative humidity was outside protocol range but did not affect study outcome."

Results:
The maximum average irritation score is 43.0, obtained 24 through 72 hours after treatment. Fluorescein staining was observed in three of three eyes at 24 hours after treatment and was not observed in any eyes at 21 days after treatment.
Under the conditions of this study, HM 4005 Antimicrobial is classified as moderately irritating.

Incidence of Irritation

Time Post Instillation	No. of Animals Testing "Positive" / No. of Animals Tested			Severity – Mean Score
	Corneal Opacity	Iritis	Conjunctivae	
1 hour	0 / 3	2 / 3	1 / 3	12.0
24 hours	3 / 3	3 / 3	3 / 3	43.0
48 hours	3 / 3	3 / 3	3 / 3	43.0
72 hours	3 / 3	3 / 3	3 / 3	43.0
Day 4	1 / 3	1 / 3	3 / 3	15.0
Day 7	1 / 3	1 / 3	1 / 3	12.3
Day 10	1 / 3	1 / 3	1 / 3	13.7
Day 14	1 / 3	1 / 3	1 / 3	13.0
Day 17	1 / 3	0 / 3	1 / 3	9.3
Day 21	0 / 3	0 / 3	0 / 3	0.0

Individual Scores for Ocular Irritation

Observations	Rabbit No. 4500-M (Male)									
	Hours After Treatment[1]					Days After Treatment				
	1	24	48	72	4	7	10	14	17	21
I. Corneal Opacity	+	1	1	1	2	2	4	4	4	0
II. Iris	0	1	1	1	1	1	1	1	0	0
III. Conjunctivae										
A. Redness	1	3	3	3	3	3	3	3	2	0
B. Chemosis	1	3	3	3	3	3	2	2	1	0
C. Discharge	2	3	3	3	1	2	2	2	1	0
D. Necrosis or Ulceration	0	0	0	0	0	0	0	0	0	0
Observations	**Rabbit No. 4506-M (Male)**									
	Hours After Treatment[1]					Days After Treatment				
	1	24	48	72	4	7	10	14	17	21
I. Corneal Opacity	+	1	1	1	0	0	0	0	0	0
II. Iris	1	1	1	1	0	0	0	0	0	0
III. Conjunctivae										
A. Redness	1	3	3	3	2	1	0	0	0	0
B. Chemosis	1	3	3	3	1	0	0	0	0	0
C. Discharge	2	3	3	3	1	0	0	0	0	0
D. Necrosis or Ulceration	0	0	0	0	0	0	0	0	0	0
Observations	**Rabbit No. 4501-F (Female)**									
	Hours After Treatment[1]					Days After Treatment				
	1	24	48	72	4	7	10	14	17	21
I. Corneal Opacity	+	1	1	1	0	0	0	0	0	0
II. Iris	1	1	1	1	0	0	0	0	0	0
III. Conjunctivae										
A. Redness	1	3	3	3	2	1	1	0	0	0
B. Chemosis	2	3	3	3	1	1	0	0	0	0
C. Discharge	2	3	3	3	1	0	0	0	0	0
D. Necrosis or Ulceration	0	0	0	0	0	0	0	0	0	0

+ denotes slight dulling of normal luster

[1]All treated eyes were washed with room temperature deionized water for one minute immediately after recording the 24-hour observation.

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 31
MRID No.: 479841-05

Reviewer: CSC and Ian Blackwell
Completion Date: January 21, 2010
Study No.: 13535-09

Testing Laboratory: STILLMEADOW, Inc., Sugar Land, TX
Author: Janice O. Kuhn, PhD, DABT

Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study was designed and performed at STILLMEADOW, Inc. and was conducted in compliance with U.S. EPA FIFRA 40 CFR 160, with the following exception: Characterization and stability information was not provided to the testing facility, as per Section 160.31(d) and 160.105(a)(b)(e).

Test Material: HM 4005 Antimicrobial
Batch #: GDG-071 / Clear water-like liquid

Dosage: 0.5 mL (applied undiluted)

Species: 3 Rabbits; New Zealand White, albino
Sex: 1 Male and 2 Females. Females were nulliparous and non-pregnant.
Age: Adult (15 weeks old)
Weight: Male: 2.175 kilograms; Females: 2.075-2.650 kilograms; initial body weight
Source: Nichols Rabbitry, Inc., Lumberton, TX
Housing: Temperature Range: 18-22°C
Humidity Range: 27-65%
Photoperiod: 12-hour light/12-hour dark cycle
Acclimation: 5 days

Summary:

1. **Toxicity Category**: IV (slightly irritating)

2. **Classification**: Acceptable

Procedure (Deviations from 870.2500):

- The laboratory reported the following protocol deviation: "Relative humidity was outside protocol range but did not affect study outcome."

Results:
Very slight erythema was present only at the Hour 1 observation. No edema was present at any observation. No other signs of irritation were observed during the study.

The Primary Dermal Irritation Index for HM 4005 Antimicrobial of 0.2 (out of a possible 8.0) was obtained from the observations at 1, 24, 48 and 72 hours, and was used to give HM 4005 Antimicrobial a descriptive rating of slightly irritating.

Under the conditions of this study, HM 4005 Antimicrobial is classified as slightly irritating to the skin.

Incidence of Irritation

Time after Patch Removal	Erythema	Edema
1 hour	2 / 3	0 / 3
24 hours	0 / 3	0 / 3
48 hours	0 / 3	0 / 3
72 hours	0 / 3	0 / 3

Individual Skin Irritation Scores

Animal No.	Sex	Erythema / Edema			
		Time After Patch Removal			
		1 hour	24 hours	48 hours	72 hours
4516	M	1 / 0	0 / 0	0 / 0	0 / 0
4517	F	1 / 0	0 / 0	0 / 0	0 / 0
4521	F	0 / 0	0 / 0	0 / 0	0 / 0
Total		2 / 0	0 / 0	0 / 0	0 / 0
Mean		0.7 / 0	0 / 0	0 / 0	0 / 0

Summary of Skin Irritation Scores[1]

	Time After Patch Removal			
	1 hour	24 hours	48 hours	72 hours
Erythema	0.7	0	0	0
Edema	0.0	0	0	0
TOTAL (PDI)[2]	0.7	0	0	0

[1]Average values for three rabbits
[2]PDI = Average Erythema + Average Edema